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THE EFFECTS OF ALCOHOL NOT INHERITED IN HYDATINA SENTA

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MANY experiments have been performed and much published concerning the effects of alcohol upon living organisms. Hodge, Calkins, Lieb, Woodruff, Estabrook, Matheny, and others, have observed its influence on the rate of growth and reproduction in certain unicellular organisms. Abbott, Hodge and others have carried on some experiments with mammals by which they have demonstrated that the resistance to certain bacterial infections is lowered by the influence of alcohol. Hunt and Woodruff found an increase of susceptibility to certain poisons in the animals subjected to alcohol. Abel and Welch have summarized in general the pharmacological action and the pathological effects of alcohol upon man and some of the other mammals.¹

Stockard has produced abnormal fish embryos and Féré has produced abnormal chick embryos by the use of alcohol, while Hodge, Newman, Sullivan and others have demonstrated the harmful influence of alcohol upon the embryos of mammals and man during pregnancy.

The evidence taken altogether with a few exceptions shows that when living organisms in any stage of their life are subjected to alcohol in appreciable quantities they are as a whole or in part unfavorably affected by it.

In nearly all of the previous work observations have been made especially upon the organisms themselves which have been directly subjected to the influence of alcohol at some stage of their life. As the harmful effects

¹ I am greatly indebted to Dr. F. E. Chidester for placing at my disposal his bibliography and notes of his forthcoming paper, "Cyclopia in Mammals."

of alcohol on the organisms subjected to its influence have been so conclusively demonstrated, it seems desirable to determine whether the offspring of alcoholic individuals in the subsequent generations are normal or show any of the weakness of their alcoholic ancestors. In other words the problem is to find out whether the descendants of alcoholic parents are in any way inferior to the normal individuals of the species and, if so, for how many generations the weakness continues.

That the parental use of alcohol in human beings affects some of the offspring in the first filial generation is undoubted by many observers, yet Pearson and Elderton have recently shown that the school children of alcoholic parents are as normal as the children of sober parents in physique and intelligence. However, the results set forth in this paper do not purport to have any relationship with the effects of alcohol upon man and his descendants.

While working with the rotifer, *Hydatina senta*, observations have been made which show that while alcohol decreases the rate of reproduction and increases the susceptibility to copper sulphate, still these harmful effects of alcohol disappear in the second generation after the alcohol has been removed from the culture water. The grandchildren show none of the alcoholic weakness of the grandparent, but are as normal as the individuals whose grandparents never were subjected to alcohol.

Hydatina senta can be readily reared and controlled in the laboratory in the manner described in a former paper. Alcohol can be added directly to the liquid medium in which the animals live. A large amount of the liquid is drawn through the mouth, indirectly by means of the pulsating bladder, into the alimentary canal, and the dialyzable parts pass through its walls into the body cavity and then finally out through the excretory ducts to the exterior of the body. In this way the animal is bathed both on the outside and on the inside of the body by the solution in which it is living. Consequently all internal parts and all

organs of the animal are subjected to whatever dialyzable chemical substance there may be in the solution.

The young females grow to maturity very rapidly and lay eggs which develop and hatch within a few hours. This extremely short life-cycle, from egg to egg in forty-eight hours, more or less, makes this animal a very favorable form with which to work. Many generations can be reared in a short time and as much information gained in a few weeks as it would require years to obtain from some of the other forms.

Experiments were first carried out to determine what influence a $\frac{1}{4}$ per cent., $\frac{1}{2}$ per cent. and 1 per cent. alcoholic solution had upon the race when it was subjected to it continuously for many successive generations. Precautions were taken to have all conditions, excluding the alcoholic conditions, in each generation exactly identical. The experiments were conducted in the same room so that the temperature was always uniform for each generation. The same amount of food culture from the same jar was always mixed with the same amount of water or with the same amounts of the various alcoholic solutions thus making the proportion of food culture to the mixture always alike. This mixture was then poured out into watch glasses and one young female rotifer put into each glass. At the end of forty-eight hours the young female had matured, layed eggs some of which had hatched, and young daughter-females would be found swimming in the dish. One of these daughter-females was isolated to start the next generation in the same manner as the mother was originally isolated. This was continued for twenty-eight consecutive generations. The twenty young females which were isolated to form the first generation were the grandchildren of the same grandmother, thus making the control, and the other three strains or groups all start originally from one female of one race. This was a very vigorous race hatched from a winter egg which was taken from a general mixed culture jar in the early spring.

Table I shows the detailed and summarized data of the

TABLE I (Continued)

Time	Control			0.25% Alcoholic Solution			0.5% Alcoholic Solution			1% Alcoholic Solution			Generation		
	Young Females Isolated	Offspring Produced	Av. No. of Offspring	Young Fe-males Isolated	Off-spring Pro-duced	Av. No. of Offspring	Young Fe-males Isolated	Off-spring Pro-duced	Av. No. of Offspring	Young Fe-males Isolated	Off-spring Pro-duced	Av. No. of Offspring			
Eve. June 2, 1911 ..	5	30	6	5	13	2.6	5	7	1.4	4	5	1.25	XV		
Eve. June 4, 1911 ..															
Eve. June 4, 1911 ..				5	43	8.6	1	0	0	3	11	3.66	5	13	2.6
Eve. June 7, 1911 ..	5	36	7.2	—	—	—	3	12	4	5	8	1.6	XVII		
Eve. June 7, 1911 ..															
Eve. June 9, 1911 ..															
Eve. June 9, 1911 ..	5	57	11.4	1	3	3	5	18	3.6	5	2	0.4	XVIII		
Eve. June 11, 1911 ..															
Eve. June 11, 1911 ..				4	26	6.5	5	6	1.2	5	5	1	4	2	0.5
Eve. June 13, 1911 ..	5	31	6.2	3	9	3	4	16	4	4	2	0.5	XX		
Eve. June 14, 1911 ..															
Eve. June 16, 1911 ..															
Eve. June 16, 1911 ..	5	21	4.2	5	19	3.8	5	10	2	4	0	0	XXI		
Eve. June 16, 1911 ..															
Eve. June 18, 1911 ..															
Eve. June 18, 1911 ..	5	91	18.2	5	38	7.6	5	20	4	5	5	1	XXII		
Eve. June 21, 1911 ..															
A.M. June 22, 1911 ..															
A.M. June 24, 1911 ..	4	40	10	4	29	7.25	5	9	1.8	5	7	1.4	XXIII		
A.M. June 24, 1911 ..															
A.M. June 24, 1911 ..															
A.M. June 26, 1911 ..	10	69	6.9	5	24	4.8	5	12	2.4	5	5	1	XXIV		
A.M. June 26, 1911 ..															
A.M. June 26, 1911 ..															
A.M. June 28, 1911 ..	5	68	13.6	5	37	7.4	4	8	2	5	7	1.4	XXV		
A.M. June 28, 1911 ..															
A.M. June 28, 1911 ..															
A.M. June 30, 1911 ..	5	79	15.8	5	14	2.8	5	8	1.6	3	1	0.33	XXVI		
A.M. June 30, 1911 ..															
A.M. June 30, 1911 ..															
A.M. June 30, 1911 ..	5	52	10.4	1	4	4	5	20	4	5	4	0.8	XXVII		
A.M. July 2, 1911 ..															
A.M. July 2, 1911 ..															
A.M. July 4, 1911 ..	5	15	3	3	1	0.33	1	0	0	3	3	1	XXVIII		
A.M. July 4, 1911 ..															
A.M. July 4, 1911 ..															
Summary	141	1,189	8.43	115	576	5.00 +	128	424	3.31	121	187	1.54 +			

observations made upon the twenty-eight generations while they were subjected to the influence of the alcohol. One can not compare strictly the number of individuals in the different generations because of the changed conditions, especially of the temperature, and in some instances the length of time between the generations. However, the ratios between the strains or groups in all the generations may be compared and will show general uniformity. The first few generations of the $\frac{1}{4}$ per cent., and the $\frac{1}{2}$ per cent. alcoholic strains show a fluctuation in the rate of reproduction above and below that of the control; but this rate of reproduction in the $\frac{1}{4}$ per cent. alcoholic strain never rises to that of the control after the sixth generation. In the $\frac{1}{2}$ per cent. alcoholic strain the rate of reproduction never rises to that of the control after the third generation. In the 1 per cent. alcoholic strain the rate of reproduction even in the first generation does not equal that of the control. The summary shows in the average number of offspring for each female that the alcoholic strains differ in the rate of reproduction according to the amount of alcohol used. The more alcohol used the lower the rate of reproduction.

Another test to show the influence of the 1 per cent. alcohol in this same series was made by removing some of the individuals from the alcoholic solution and placing them in a 1/14,000 G. M. copper sulphate solution² and comparing the resisting power, or the ability to live, of this strain with that of the control when both were subjected to the copper sulphate solution. Table II shows the detailed data and Table III shows the summary. In the control 96.8 per cent. of the individuals lived forty-eight hours and produced young, while only 15 per cent. of the individuals taken from the 1 per cent. alcoholic strain in the XIII–XV generations lived forty-eight hours and produced young. This shows that the susceptibility to copper sulphate is greatly increased by the alcohol.

² Various solutions of copper sulphate were tried and the one employed was selected because it appeared to be of the maximum strength which the control could withstand.

TABLE II

SHOWING THE LOWER RESISTING POWER TO COPPER SULPHATE OF FEMALES REARED THIRTEEN TO FIFTEEN GENERATIONS IN A 1 PER CENT. ALCOHOLIC SOLUTION, AND ALSO SHOWING THAT THE RESISTING POWER HAS BEEN REESTABLISHED IN THE SECOND GENERATION AFTER THE ALCOHOL HAS BEEN REMOVED
(See Table III for Summary)

Experiment		Young Females Isolated	$\frac{1}{14000}$ G. M. Copper Sulphate Solution		
			24 Hours	36 Hours	48 Hours
1	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
	1 % alcohol	5	Alive	All dead	
2	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
	1 % alcohol	5	2 dead	All dead	
3	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
4	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
5	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
6	Control	5	Alive	Alive	Alive + young
	Second water generation	5	Alive	2 dead	3 alive + young
	1 % alcohol	5	Alive	4 dead	All dead
7	Control	5	Alive	Alive	Alive + young
	Second water generation	5	Alive	Alive	Alive + young
	1 % alcohol	5	Alive	4 dead	All dead
8	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
	1 % alcohol	5	3 dead	4 dead	All dead
9	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
	1 % alcohol	5	Alive	Alive but in poorer condition. Fewer young	1 dead. Others nearly dead Fewer young
10	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
	1 % alcohol	10	5 dead	5 dead, fewer young	6 dead + fewer young
11	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
	1 % alcohol	10	All dead		
12	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
	1 % alcohol	10	Alive	6 dead	6 dead + young
13	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
	1 % alcohol	10	3 dead	5 dead	7 dead + young
14	Control	20	4 dead	4 dead	4 dead + young
	Second water generation	20	3 dead	3 dead	3 dead + young
	1 % alcohol	20	19 dead	All dead	
15	Control	10	Alive	Alive	Alive + young
	Second water generation	10	6 dead	6 dead	6 dead, young
	1 % alcohol	10	All dead		

On July 1, these four strains of rotifers were carried to Woods Hole, Mass. Owing to the high temperature difficulty was experienced in growing proper food cultures and consequently by July 4 many of the animals had died and those that had survived were in a very bad condition and had very few offspring. It is interesting to note that more of the animals in the alcoholic strains died at this time in the twenty-eighth generation than in the control strain. The experiments were discontinued on account of these unfavorable conditions.

TABLE III
SHOWING SUMMARY OF TABLE II

	Copper Sulphate Solution					Per Cent. Living at End of 48 Hours
	No. of Young Females Isolated	No. Died in 24 Hours	No. Died in 36 Hours	No. Died in 48 hours	No. Living at End of 48 Hours	
Control	125	4	4	4	121	96.8
Second water generation . . .	125	9	11	11	114	91.2
1 % alcohol . . .	100	52	78	85	15	15

The data in these three tables seem to show that alcohol from $\frac{1}{4}$ per cent. to 1 per cent. has a decided influence in lowering the rate of reproduction and also in lowering the power of resisting copper sulphate in the individuals of the 1 per cent. alcoholic strains. Presumably the resisting power to copper sulphate of the $\frac{1}{4}$ per cent. and the $\frac{1}{2}$ per cent. alcoholic strains was similarly lowered, but this was not determined.

This decrease of the reproduction rate and the increased susceptibility to copper sulphate can be considered as an indication that the "general vitality" of the race had been lowered in that the individuals were much inferior to the control individuals in their ability to cope with adverse conditions and to leave offspring with which to continue the race.

Since it is shown that alcohol decreases the "general vitality" of these animals the condition of their offspring now remains to be considered. Table IV gives the de-

tailed and summarized data of experiments showing the comparative reproduction rates of the offspring from the three alcoholic strains between generations XI and generation XXIII, special emphasis being laid upon the offspring from the parents in the 1 per cent. alcoholic strain, and the reproduction rate of the control or normal strain. Young females were isolated from the three alcoholic strains placed in media containing no alcohol and reared two generations parallel to the alcoholic strains. In this way they were under exactly the same conditions as the control strain. The isolations of young females from the $\frac{1}{4}$ per cent. and the $\frac{1}{2}$ per cent. alcoholic strains were discontinued after a few experiments and the time devoted to experiments with the 1 per cent. strain. As this strain in Table I showed the lowest reproduction rate and was decidedly susceptible to the influence of copper sulphate, it was assumed to have suffered the most of any of the three strains subjected to alcohol and therefore was considered to be the most favorable to show the effects of alcohol upon the offspring. For the sake of clearness all the data are so arranged in Table IV as to show the three rates of reproduction in the same generation, of the control, alcoholic strains, and the alcoholic strain with the alcohol removed. In the first water generation the young females were isolated from the preceding alcoholic generation soon after hatching and reared in media containing no alcohol. Thus the formation of the egg from which each young female hatched and all the embryonic development occurred in the alcoholic solution. After being transferred to culture water lacking alcohol they grew to maturity and reproduced. This generation is called Water Generation I. Some of the young daughter-females from Water Generation I were isolated to form Water Generation II.

In comparing the rates of reproduction in the Water Generation I with the rate of reproduction in the same generation of the alcoholic strains it is seen that in all cases the rate of reproduction is higher in the Water

TABLE IV (Continued)

Experiment	Time	Control				0.25 Per Cent. Alcohol				0.5 Per Cent. Alcohol				1 Per Cent. Alcohol				Alcoholic Generations	Water Generations																													
		Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated			Offspring Produced	Average No. of Offspring																											
6	Eve. June 14, 1911	{ 5	31	6.2	3	9	3	—	—	4	16	4	—	—	—	—	4	2	0.5	21	26	1.23	XX	I																								
7	Eve. June 16, 1911	{ 5	21	4.2	5	19	3.8	—	—	5	10	2	—	—	—	—	4	0	0	29	126	4.34	XXI	II																								
8	Eve. June 18, 1911	{ 5	91	18.2	5	38	7.6	—	—	5	20	4	—	—	—	—	5	5	1	2	14	7	XXII	I																								
	A.M. June 22, 1911	{ 5	51	10.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9	90	10	XXIII	II																								
	A.M. June 23, 1911	{ 5	80	16	4	29	7.25	—	—	5	9	1.8	—	—	—	—	5	7	1.4	7	105	15	XXIII	II																								
	A.M. June 24, 1911	{ 4	40	10	4	29	7.25	—	—	5	9	1.8	—	—	—	—	5	7	1.4	13	34	2.61	XXIII	I																								
	A.M. June 24, 1911	{ 10	69	6.9	5	24.8	—	—	—	5	12	2.4	—	—	—	—	5	5	1	29	258	8.89	XXIV	II																								
	Summary	{ 39	405	10.38	28	190	6.78	16	112	7	32	139	4.34	23	147	6.39	37	62	1.67	56	186	3.32	XI, XII, XIV, XVI, XVIII, XX, XXII, & XXIII, XXIV, XXV, XXVII, XXIX, XXI, XXII, XXIII & XXII.	I																								
	Summary	{ 44	348	7.90	29	103	3.55	15	105	7	33	65	1.96	18	134	7.44	31	31	1	92	708	7.69	XIX, XXI, XXII, XXIII & XXII.	II																								

Generation I than it is in any of the corresponding alcoholic strains. However, it never reaches that of the control. In the Water Generation II the rate of reproduction is much higher than it is in Water Generation I and *equals* the reproductive rate of the control. This demonstrates that one of the ill effects of alcohol is partially eliminated in the first generation and is entirely eliminated in the second generation after the alcohol has been removed.

Very probably the rate of reproduction of Water Generation I, which is lower than that of the control, is due to the fact that the females in their embryonic development were subjected to the influence of alcohol from which they never fully recovered after they were transferred to water solutions containing no alcohol. They were thus perhaps influenced while in the growth and maturation stages of the egg inside the body of the mother or, after the egg was laid in the alcoholic solution, in the embryonic stages which occurred inside the egg membrane before hatching. Soon after hatching the females were put into the solutions free from alcohol.

This transmission of a low reproduction rate to Water Generation I is in reality not a hereditary transmission of a characteristic, but is probably the result of the direct influence of the alcohol upon the mother during her embryonic development.

Tables II and III show the effect of copper sulphate upon Water Generation II. Of the individuals tested 91.2 per cent. lived forty-eight hours and produced young in the copper solution. This is only about 5 per cent. less than the number of individuals of the control living and producing young for the same length of time in the copper solution. It can be concluded from these observations that the resisting power to copper sulphate has been restored practically to normal and that these individuals are no more susceptible to its influence than the individuals of the control.

Billings states to the Committee of Fifty in his report,

which is based on over five thousand reports of cases of insanity, that: "Inherited tendency to insanity, due to the use of liquor by parents, is reported in one hundred and twenty-two cases . . . while six cases were ascribed to the intemperance of the grandparents. These statistics must be received with caution as showing possibilities rather than as definite evidence. To prove that the insanity of one generation is due to alcoholic excess of a previous generation, and is not merely a coincidence, requires that other causes of degeneration shall be carefully studied, and duly allowed for."

It is, however, evident from the six cases reported that some, at least, of the medical examiners believe in the transmission of alcoholic weaknessess from grandparents to grandchildren.

Bunge, from an investigation extending over two thousand families, found that chronic alcoholic poisoning in the father was the chief cause of the daughter's inability to suckle and that this inability was not usually recovered from in subsequent generations. These results have been severely criticized by Bluhm and their validity questioned.

Mariet and Cambemale gave considerable quantities of alcohol to a female dog during the last week of her pregnancy. She gave birth to a litter of seven puppies, of which four were dead, two apparently healthy but mentally backward, and one, No. 7, both physically and mentally backward. No. 7 was a female and grew to maturity free from the influence of alcohol and mated with an apparently healthy dog. All of the puppies of her first litter were abnormal to such a degree that they were considered worthless. One had club feet and a clefted palate, another had a conspicuous ductus Botalli, and another developed muscular atrophy in its hind legs.

If these observations and interpretations are correct they may demonstrate either the same fact that is shown in Water Generation I of Table IV, namely, that when a mother is subjected to the influence of alcohol during her

own embryonic development she shows some sign of weakness at her period of reproduction, or, that the grandchildren are affected by the influence of alcohol upon their grandparents. However, only one experiment alone like the above is not sufficient to prove anything, and furthermore, Hodge in speaking of dogs says: "We do not attach much importance to the greater percentage of deformity, since this is of somewhat common occurrence in kennels."

If the transmission of an alcoholic weakness to subsequent generations is possible in any living organism, it ought to be actually demonstrated in some manner, but if it is a delusion, the sooner it is dispelled the better. These experiments with *Hydatina senta* are an attempt to determine, in one race of animals only, whether certain alcoholic weaknesses are truly hereditary and the evidence found is negative.

It by no means follows that these results would be found to be true in man. Alcohol primarily affects the nervous system and may have a very different action on the highly organized nervous system of man than it does on the lowly organized *Hydatina*, whose nervous system is extremely simple. Furthermore, the germ substance in man is probably very different from the germ substance in the rotifer and alcohol might have a very different effect upon it.

SUMMARY

1. Four strains of parthenogenetic rotifers originally descended from the same female were observed throughout twenty-eight successive generations. One strain was kept as a control and the other three strains were kept in a $\frac{1}{4}$ per cent., a $\frac{1}{2}$ per cent. and a 1 per cent. solution of alcohol. The rate of reproduction was lower in the alcoholic strains than in the control and it was proportionally lowered according to the amount of alcohol used.

2. The individuals of the 1 per cent. alcoholic strain in the XI-XV generations showed a decidedly increased susceptibility to copper sulphate.

3. When the alcohol was removed in generations XI-XXII, the rate of reproduction increased noticeably in the first generation and in the second generation the reproduction rate equaled that of the control.

4. Individuals of the second generation after the alcohol had been removed were no more susceptible to copper sulphate than individuals which had never been subjected to alcohol.

5. The general conclusion is that alcohol in $\frac{1}{4}$ per cent., $\frac{1}{2}$ per cent., and 1 per cent. solutions is detrimental to this race of rotifers when it is subjected to it continuously for many generations. The weaknesses developed by the parental use of alcohol are partially eliminated in the first generation after the alcohol has been removed, and practically completely eliminated at the end of the second generation after the alcohol has been removed. In other words, the grandchildren possess none of the defects caused by alcohol in the grandparents.

6. These results in general show that alcohol in the percentages used affects only the somatic tissues of the animal, and if they are subjected to its influences indefinitely, generation after generation, the race would probably become extinct because of its "lowered resistance power" to unfavorable conditions. However, if the alcohol is removed it is possible for the race to recover and to regain its normal condition in two generations, thus showing that the germ substance is not permanently affected by the alcohol.

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